UNUSUAL POLLEN DIMORPHISM IN RONDELETIA ANGUILLENSIS (RUBIACEAE)

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Long-styled plants of *Rondeletia anguillensis* bear a mixture of three- and four-colpate pollen, whereas short-styled plants bear only three-colpate grains. Short-styled plants have smaller pollen grains with lower pollen stainability than long-styled plants.

In the preceding paper (Howard & Kellogg, 1987) we described *Rondeletia anguillensis,* a new species collected on the Caribbean island of Anguilla in 1985. The plants were clearly distylous, a condition common in the Rubiaceae. Measurements confirmed that short-styled plants had notably longer corolla tubes than long-styled ones (4–5.7 vs. 3.2–3.6 mm, respectively); this difference in corolla size has been reported for other distylous plants (Ganders, 1979). In the process of preparing the description, we discovered that the pollen of the two stylar forms was more strongly dimorphic than is commonly the case in the family. We report our results here in the hope of stimulating further collecting and investigation of the phenomenon.

METHODS

Our observations were based on material from three collections, two short-styled plants (*Howard & Kellogg 20105,* A; *Proctor 18571,* A) and one long-styled one (*Howard & Kellogg 20103,* A) (for full specimen citations, see Howard & Kellogg, 1987). After preparing SEM photographs of pollen from each of the two forms, we continued investigations with the light microscope. Pollen was stained overnight with cotton blue in lactophenol. For each plant the diameters of 200 pollen grains from a single flower were measured and averaged. Pollen stainability was calculated from more than 200 grains for each flower observed (four flowers for each of the Howard & Kellogg collections, one for the Proctor collection). For each of these nine flowers, the number of colpi was recorded for the first 200 grains from which it could be determined.

RESULTS AND DISCUSSION

The photographs in the FIGURE show that the long-styled plant has a mixture of three- and four-colpate pollen (a, b), whereas the short-styled plant bears

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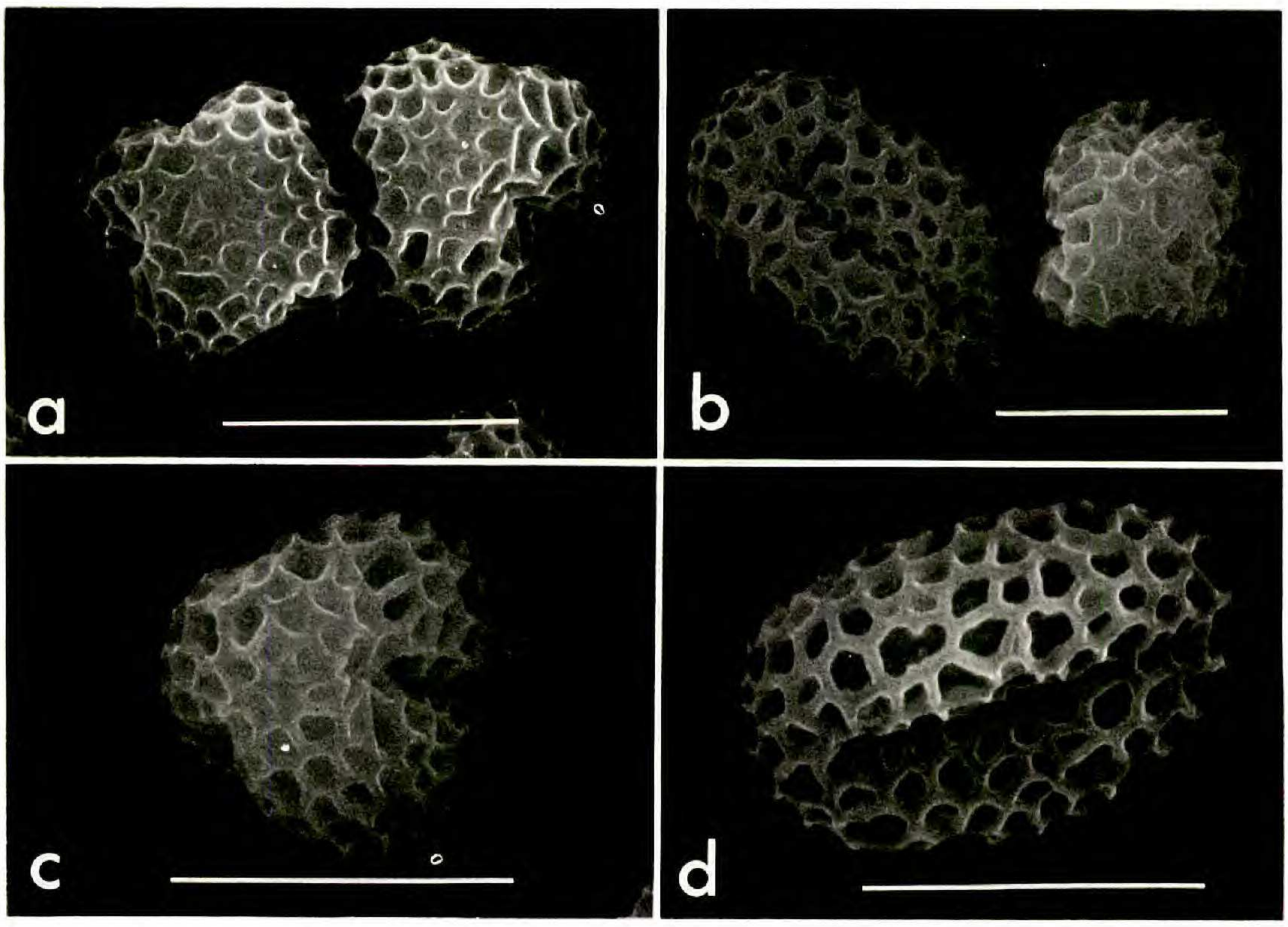
Journal of the Arnold Arboretum **68**: 133–136. January, 1987.



Rondeletia anguillensis, nonacetolyzed pollen (scale bars = 10 μm): a, b, long-styled plant, *Howard & Kellogg 20103*; c, d, short-styled plant, *Howard & Kellogg 20105.*

consistently three-colpate grains (c, d). Also, pollen from the short-styled plant appears to be slightly smaller than that from the long-styled one.

These observations were confirmed by light microscopy, the results of which are summarized in the TABLE. Pollen size and stainability were virtually identical for the two short-styled plants, despite the fact that they were collected 26 years apart and at different localities on the island. Unfortunately, we had only one long-styled plant, and that had very few nearly mature buds from which to take pollen, so we cannot be certain that the pollen dimorphism is characteristic of the species. There is still the possibility that *Howard & Kellogg 20103* is simply an anomalous plant.

These preliminary results show some surprising differences between the two style forms. Statistical comparison of the mean sizes of pollen of the two Howard & Kellogg collections produces $t = 8.96$, df = 398, a difference significant at $p \ll 0.005$. However, the short-styled plants have the smaller pollen, contrary to the condition in most other distylous plants. Ganders (1979) reported pollen of short-styled plants to be smaller than that of long-styled ones in *Fauria crista-galli* Makino (Menyanthaceae); this was apparently the first report of such a size relationship.

Variation in pollen stainability has been reported by Ornduff (1980) in populations of *Hedyotis caerulea* (L.) Hooker. He reported variation both between and within short- and long-styled plants, and variation between and within years. There may be similar variability in *Rondeletia anguillensis,* but its significance is unclear.

Variation in pollen size, colpus number, and stainability for three plants of Rondeletia anguillensis.

Specimen	Number of colpi	Size (μm) Range	Mean	SD	Stainability (%)
Short-style					
Howard & Kellogg 20105	3*	11–15	13	0.75	72, 76, 93, 94
Proctor 18571	3	11–15	13	0.76	76
Long-style					
Howard & Kellogg 20103	3, 4	12–15	14	0.61	99, 99, 99.5, 99.5

*One 4-colpate grain observed out of more than 900. Most probably contamination.

The variation in colpus number is interesting for two reasons. First, morphological differences between long- and short-style pollen are usually subtle, particularly in the Rubiaceae. Major differences in shape have been reported for *Lithospermum* L. (Boraginaceae; Johnston, 1952), and differences in exine sculpturing occur in the Plumbaginaceae (Baker, 1966). The only really marked difference in pollen morphology for distylous Rubiaceae was reported by Baker (1956) for *Rudgea jasminoides* (Cham.) A. Rich., in which the pollen of long-styled plants was smooth and that of short-styled ones was spiny.

Second, four-colpate pollen is one of the major characters used by Borhidi and colleagues (1980, 1981) in distinguishing *Rondeletia* L. and the segregate genera *Roigella* Borhidi & Zequeira, *Neomazaea* Urban, and *Acuneanthus* Borhidi, Jarai-Komlodi, & Moncada; pollen of *Rondeletia* and *Acuneanthus* is three-colpate, while that of the other two genera is four- or five-colpate. The variation we have found in this character casts some doubt on its usefulness at the generic level. Its variability within species should perhaps be investigated more fully before it is relied upon for major distinctions among groups.

Four flowers from the long-styled plant were scored for percent of three-colpate pollen. The percentages were 47, 51, 59, and 62. The pooled $\chi^2 = 7.0$, indicating that the ratio of three- to four-colpate pollen was significantly different from 1:1 ($p < 0.01$). However, a test for homogeneity of χ^2 values among the flowers showed a significant lack of homogeneity, so pooling the values may not be justified. If χ^2 values are calculated separately, values for two of the flowers are not significantly different from a 1:1 ratio, whereas values for the other two are. If we assume that the two pollen morphs would indeed appear in equal proportions if the sample were sufficiently large, then we could explain the observed variation in pollen morphology by a one-locus gene with two alleles, one of which conditions for three colpi and the other for four. Under this explanation, the long-styled plant is heterozygous, the (haploid) pollen grains therefore being half three- and half four-colpate, whereas the short-styled plants are homozygous. If this proves to be the case, it would be an interesting parallel with the gene for distyly itself, which in all reported cases is also one locus with two alleles and complete dominance (Ganders, 1979).

One style morph is then homozygous recessive (ss), while the other is heterozygous (Ss). The homozygous dominant does not occur because of self-incompatibility of the heterozygotes. Although the short-styled plants are the heterozygotes in most species, long-styled heterozygotes have been reported (Ganders, 1979). It is conceivable that the gene for pollen shape could be linked with the gene for distyly to produce the pattern we have observed in *Rondeletia anguillensis.*

ACKNOWLEDGMENTS

This study was carried out with support from National Science Foundation grant BSR 83-07701.

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